Antagonists of protein–protein interactions

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Protein–protein interactions are often attractive, but not straightforward, targets for disease therapy. Two strategies for identifying inhibitors of these interactions, peptide phage display and high-throughput screening, have recently shown new promise.

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Introduction

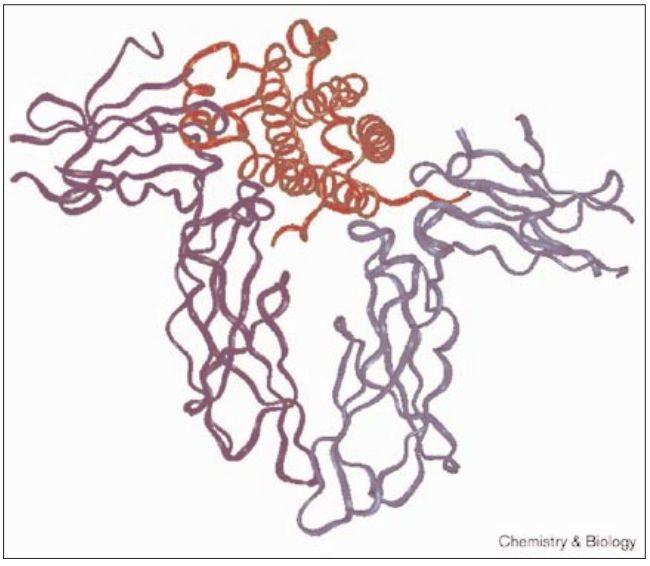
The work of a living cell is performed largely by proteins, sometimes alone but often in concert with partners. Much of this activity is mediated by enzymes such as kinases, proteases or glycosylases. However, many regulated processes are initiated or inhibited through specific protein–protein complex formation. Cytokine signaling and transcription are examples. With the availability of complete genome sequences, it has been estimated that the number of interacting protein pairs in *Escherichia coli* may be 6800 or more, and in *Saccharomyces cerevisiae* may exceed 45,000 [1]. No doubt in humans the number will be much higher, and many of these interactions will ultimately become pharmaceutical targets. Protein–protein interactions are generally quite specific, and much can be learned about their function through genetic and biochemical means. Despite the availability of such detailed information, these interactions are considered difficult small-molecule targets. This review will outline some of the difficulties and summarize the results that have emerged so far in the field, without attempting to be exhaustive. In particular, I will not discuss G-protein-coupled (7 TM) receptors or small docking modules.

some recent advances). Instead, I will discuss systems in which both binding partners are fairly large soluble proteins (or soluble, independently folded domains of a protein), with binding contacts spread over a surface. Often this type of binding surface includes discontinuous parts of the protein sequence, sometimes from more than one protein domain. The complex of human growth hormone and its receptor is a good example of this type (Figure 1) [5,6].

What is a small molecule, and why are protein–protein targets so hard?

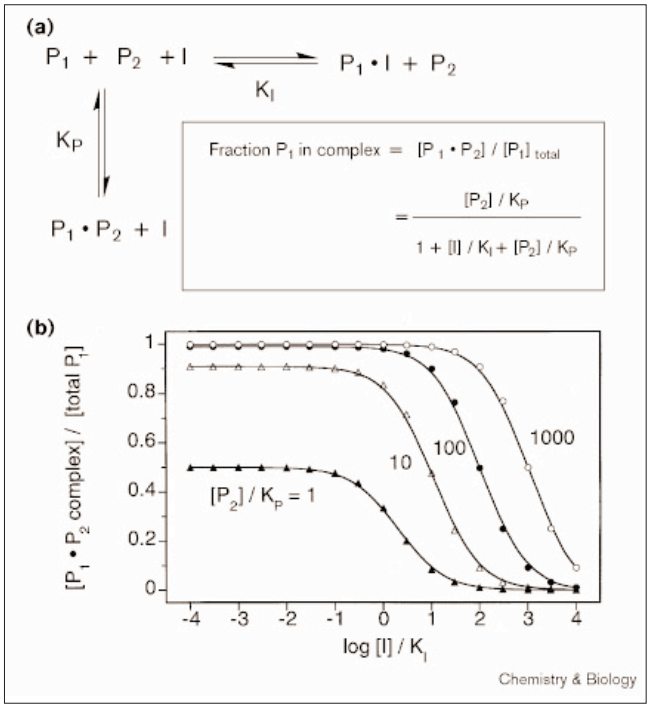
When discussing small molecules as potential pharmaceutical agents, the ultimate goal is *in vivo* efficacy, and developing such molecules is far from trivial. Ideally, one wants not only high potency against the target protein, but also low toxicity, few side effects and good bioavailability, preferably oral bioavailability. Some efforts have been made to analyze databases of existing drugs (about 5000 compounds) in order to define those properties that make a molecule ‘drug-like’ [3,7–10]. Very generally, molecular weights less than about 500 are preferred, and molecules that are either extremely hydrophobic or extremely hydrophilic are not considered optimal. There are very few reports of drug-like molecules disrupting protein–protein interfaces.

Figure 1



Complex of human growth hormone (red) and its receptor (blue) [75].

Figure 2



(a) Competition binding equilibrium. P_1 and P_2 are proteins that associate as the complex P_1•P_2. I is an inhibitor that binds to P_1. The boxed equation relates the fraction of P_1 in the complex to the concentrations and dissociation constants of the competitors P_2 and I. **(b)** The fraction of P_1 in the complex is plotted as a function of added inhibitor (relative to its dissociation constant K_I) for several concentrations of P_2.

Historically, many efforts to discover low molecular weight drug leads have focused on inhibiting enzymes implicated in a particular disease state or on modifying known, bioactive small molecules (neurotransmitters, for example). There are some strategic advantages to these choices. Most obvious of these is that knowing even one substrate of an enzyme (or the structure of a small-molecule ligand) provides information about what kinds of other molecules might bind to the target protein, facilitating further design. This information is not obtained automatically when protein-binding partners are identified. Instead, mutagenesis experiments or structural studies identify protein contact regions and pinpoint residues most critical to binding. At this stage, many different strategies are considered for transfer of epitopes to smaller entities (peptide or nonpeptide). As yet, there is no general solution to this transfer problem. Alternatively, one may screen compound libraries directly for small-molecule inhibitors, or in the case of peptides, select ligands using phage-display methods.

It is important to realize that small-molecule discovery against protein–protein targets is genuinely difficult, but not for one of the reasons commonly assumed. It is often stated that a small molecule would require the affinity of the protein (frequently subnanomolar) to successfully compete for binding to its site [11,12]. This assumption is false. For the simplest case of inhibitor I competing with protein P_2 for binding to protein P_1, the equilibria are shown in Figure 2a. The fraction of P_1 bound to P_2 can be expressed in an equation analogous to that describing competitive enzyme inhibition (Figure 2a). Plotting this fraction as a function of added inhibitor yields a typical displacement curve, as shown in Figure 2b for several concentrations of the protein competitor P_2. How well the ligands compete for P_1 is determined not by their relative binding constants but by the ratio of each ligand's concentration to its own binding constant. This is true for all values of K_P and K_I (Figure 2). In other words, even a protein with a picomolar binding constant will be unable to compete with a weakly binding small molecule if the concentration of the protein is very low and the small-molecule concentration is relatively high. Of course, the limited solubility or bioavailability of many small molecules and their potential toxicity are often practical reasons to prefer higher affinity drug leads.

The sensitivity of protein–protein interactions *in vivo* will probably vary depending on both the levels of the proteins present and how they are distributed in the organism, and it may be critical to target the correct protein of the pair for maximal effect. Intriguingly, analysis of signaling cascades has indicated that the net output of a network may be more sensitive to small changes in effector concentration (or perhaps percent inhibition) than an individual protein component [13,14]. In other cases, a system may respond over a very wide range of effector concentrations [15], requiring

more complete titration by an inhibitor. The prospect for success in targeting a protein–protein interaction depends, therefore, on the biology of the system.

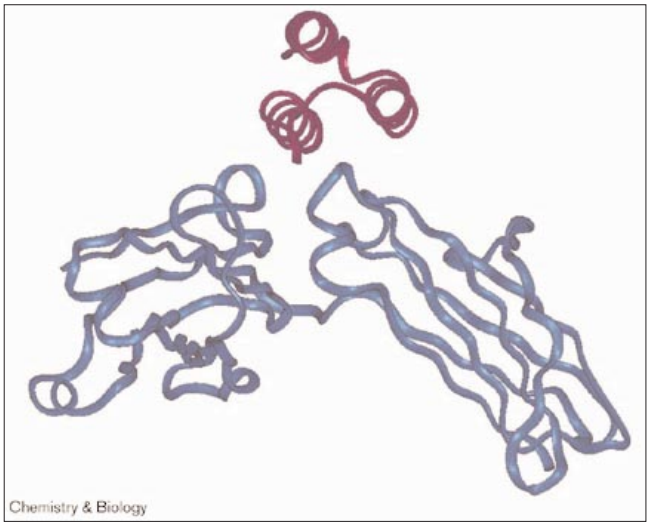
Finally, understanding molecular recognition of protein surfaces is a challenging physical chemistry problem. The crystal structures of protein–protein complexes have been surveyed to identify any features common to the contact surfaces [16–18]. A 'typical' interface buries ~1600 Å^2 of protein surface area (~170 atoms); this area is evenly divided between the two components, consistent with the approximate flatness of protein interfaces [18]. Contact atoms include those from both sidechains and the main-chain. The residue composition of interfaces is not greatly different from that of protein surfaces, although aromatic residues and arginine appear somewhat more frequently [17,18]. Burial of a hydrophobic surface can drive complex formation [19], but hydrogen bonds, salt bridges and water molecules occur in many interfaces.

It is unlikely that every part of a protein surface is suited to binding small molecules, and considerable effort has been made to identify those ligand sites that may be present. These efforts include computational searches for surface pockets in protein crystal structures ([20] and references therein), crystallization of proteins in multiple organic solvents [21], and nuclear magnetic resonance (NMR)-based methods for screening libraries of small organic compounds [22–25]. These experimental methods are capable of identifying very low affinity ligands (K_d > 1 mM) and the location of the protein-binding site. Fesik and coworkers [22] have used such a procedure, which they call 'SAR by NMR', to discover molecules that occupy adjacent pockets on a target protein. Two low-affinity ligands then can be linked to yield a high-affinity ligand (K_d < 100 nM) [22,23]. These methods have most often succeeded when targeting enzymes or proteins with known small-molecule-binding sites (e.g. the FK506-binding protein, FKBP [22]); in principle, however, they might be used to explore protein–protein recognition surfaces.

Scaffold reduction and epitope transfer: proteins to miniproteins

An attractive way to develop molecules that antagonize a protein–protein interaction would be to reproduce the essential features of one of the protein partners in a much smaller form. The critical assumption is that only a subset of protein residues make energetically significant contacts in the complex; the remainder are either completely expendable, or they are needed to stably structure those residues in contact. This idea has been tested by combining structural information, mutagenesis data and phage display to systematically minimize small proteins [26]. These efforts yielded peptides of about half the original size that retained nearly all of the original binding affinity.

Figure 3



Model of the complex of Protein A Z-domain (purple) and IgG Fc [27].

An example of this approach is the reduction of the 59-residue Z-domain from Protein A [27]. Z-domain is a three-helix bundle that binds to immunoglobulin G (IgG_1) with a dissociation constant of 10 nM (Figure 3). Although one of the helices makes no direct contact in the complex crystal structure, deletion of this helix abolished binding. Selection for variants that were capable of binding (using phage-display methods) identified 12 mutations (of the 33 remaining residues) that, when combined, restored much of the original affinity for IgG (K_d = 43 nM). These mutations increased the helicity (i.e. the structural stability) of the miniprotein; some improvements were also made in the IgG contact surface [27]. The stability and affinity of the minimized protein were further improved by adding a disulfide, resulting in a highly structured molecule that faithfully presents the essential features of the original domain [28].

A different design strategy has been used to transfer key binding contacts from the T-cell surface glycoprotein CD4 onto a much smaller scorpion toxin scaffold [29]. The toxin fold is exceptionally stable, with three disulfides in 31 residues, and is therefore very tolerant of substitutions. Thus, CD4 residues could be introduced into spatially appropriate positions in the toxin without disrupting its fold. In a first step, 11 mutations were made, and four residues were deleted from the termini. This toxin variant competed with CD4 for binding to HIV-1 gp120 (IC_{50} = 40 μM). The affinity of the initial variant was improved about 100-fold by combining alanine substitutions that individually enhanced binding of the miniprotein, and by repositioning some of the putative binding contacts. Importantly, the single most critical residue in CD4 (Phe43) is absolutely critical in the toxin, suggesting that the miniprotein binds to gp120 in the

same way as the larger protein does. The final 28-residue, mini-CD4 binds to gp120 about 100-fold more weakly than soluble CD4 [29].

A designed mimic of interleukin-4 (IL-4) illustrates again the principle of protein minimization [30]. IL-4 is a four-helix bundle cytokine. One face of the bundle displays the major binding contacts for the receptor IL-4Rα; these are primarily segregated within two of the four helices. Based on superposition of the structures of IL-4 and the GCN4 coiled coil (a two-helix structure), eight residues from IL-4 were transferred onto the surface of GCN4. The resulting mimic was found to bind weakly to IL-4Rα (K_d =26 μM for the mimic versus 1.4 nM for IL-4) [30]. Thermal denaturation studies indicated that the GCN4 mimic was partially unfolded. Stability and affinity could be improved (3–5-fold) by addition of a disulfide to cross-link the dimeric coiled coil [30].

These three examples illustrate that, beginning with some structural and mutagenesis data, it is possible to evolve or design smaller proteins that reproduce the binding properties of their larger parents. Although the CD4 and IL-4 miniproteins do not have the full affinity of the original proteins, the results are nonetheless intriguing because little optimization was required. It will be interesting to see whether further improvements in binding can be made, and to what extent miniproteins can be further minimized.

Proteins to peptides (or to peptidomimetics)

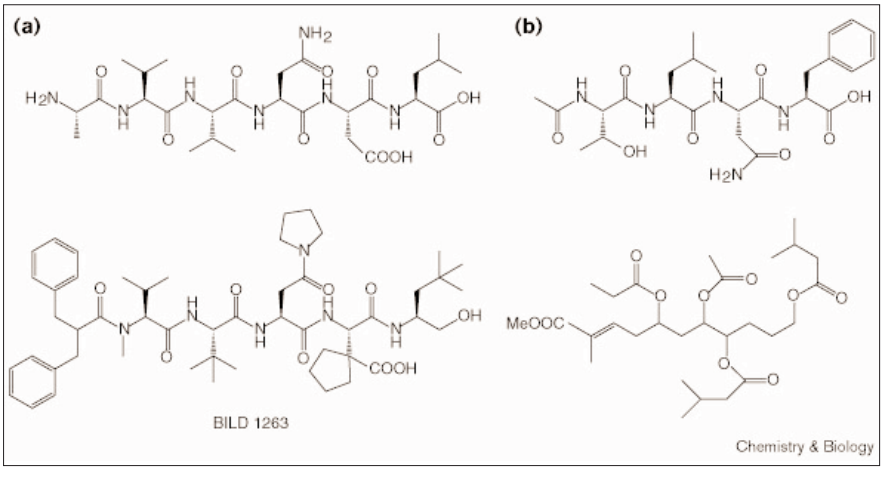
Although proteins may be reduced substantially in size as described above, the reduced proteins are still quite a bit larger than typical small-molecule drugs. It would be advantageous, when possible, to skip protein minimization and go directly to a much smaller peptide or peptidomimetic. In certain cases, this approach has proven remarkably successful. Unfortunately, it is not always so simple. Protein-binding epitopes are seldom concentrated in a short linear sequence or even in a single secondary structural element. Furthermore, short peptides are generally not structured as they might be when embedded within a protein domain. These factors combine to limit the information that may be transferred from a protein into a related peptide.

Despite these considerations, there have been many reports of short peptides (10–20 amino acids taken from a protein sequence) that compete with the protein for target. In particular, disulfide constraint of protein surface loops is commonly reported to yield peptide mimics. Such reports should be viewed with caution, however. We have tried (without success) to reproduce several published claims of peptide mimicry of a large protein domain. It requires some care to develop assays that work well with peptides and small molecules, and it is important to confirm any apparent 'hits' in secondary assays. As a general rule with peptide inhibitors: if it looks too good to be true, it probably is!

With other inhibitors the mechanism of action may be different than expected. Peptidomimetics designed to inhibit CD4 binding to HIV-1 gp120 [31] are an example of this type. The original mimetic was based on the major gp120-binding loop of CD4, and an IC_{50} of about 10 μM was reported (on the basis of two cell-based assays). It was later found, however, that a closely related mimetic did not block gp120 binding to soluble CD4 in a competition assay [32]. These molecules, therefore, cannot be considered true CD4 mimics.

By contrast, the interactions between integrins and cell adhesion molecules (CAMs) can generally be blocked using short peptides and by nonpeptide mimics (e.g. [33,34]).

Figure 4



Subunit interface inhibitors. **(a)** Peptide (top) and peptidomimetic (bottom) inhibitors of herpes virus ribonucleotide reductase. **(b)** Peptide (top) and natural product (bottom) inhibitors of HIV protease.

Recently, it has been shown that an explicit connection can be made between peptide structure–activity relationships and protein mutagenesis data for vascular CAM (VCAM) [35]. The crystal structure of a two-domain fragment of VCAM was used to design eight-residue cyclic peptide inhibitors intended to mimic the conformation of the protein-binding loop. NMR structures of two peptides were consistent with this design. Substitutions were made in the cyclic peptide to match mutations that had been examined previously in the protein. The changes in peptide IC_{50} values correlated with those observed for VCAM mutants, suggesting that the peptides and VCAM bind in the same way. Importantly, substitutions that increased inhibitory potency in the peptide suggested new VCAM mutations that improved its affinity for the integrin $\alpha_4\beta_1$ [35]. This study demonstrates that a binding epitope at a protein–protein interface may be transferred, intact, to a peptide.

Dimerization inhibitors

Enzyme dimerization interfaces appear to be another class of interaction that can be disrupted using short peptides derived from the protein [36]; this strategy has been most frequently used to inhibit viral enzymes. It was discovered in 1986 that the heterodimeric ribonucleotide reductase from herpes virus could be inhibited by short peptides derived from the carboxyl terminus of the smaller R2 subunit [37,38]. This carboxy-terminal segment anchors the R2 subunit to the larger R1 subunit, wedging between two helices on the surface of R1 [39]. Extensive analog synthesis led to the more hydrophobic and much more potent (IC_{50} = 0.3 nM) peptidomimetic BILD 1263 (Figure 4a) [40].

HIV protease is another enzyme that can be inhibited with peptides derived from the subunit interface. In this case, the enzyme is a homodimer, and the interface is a four-stranded antiparallel β sheet, with strands from the amino- and carboxy-terminal segments of each monomer [41,42]. The carboxyl termini provide the two central strands of the sheet and are flanked by the amino termini. A tetrapeptide derived from the carboxyl terminus was reported to inhibit the enzyme with a K_i of 45 μM (Figure 4b); kinetic and sedimentation analyses were consistent with inhibition by dissociation of the protease dimer [43]. This report has prompted many additional studies (reviewed in [36]). Of particular interest is a recent report that simplified analogs of the marine natural products didemnaketals A and B inhibit HIV protease [44]. Seven of the eight possible diastereomers of the didemnaketal sidechain fragment (Figure 4b) gave K_i values ranging from 2 to 30 μM. Significantly, kinetic analysis supported the dissociative mechanism [44]. It is tempting to speculate that the natural product may be a mimic of the protease β strand.

The inhibitory potency of simple linear peptides suggests that the interfaces of dimeric enzymes may differ structurally from other interfaces. It has been noted that the surface buried in oligomer interfaces is unusually hydrophobic [16,18]. Furthermore, for HIV protease, the carboxy-terminal tetrapeptide contributes a large fraction (~50%) of the total surface area buried in the dimer interface [42]. For both of the examples described in the preceding paragraph, the inhibitory peptide is expected to be significantly buried in the complex with target, rather than binding to a more exposed surface. All of these factors may combine to make subunit interfaces amenable to small-peptide inhibition.

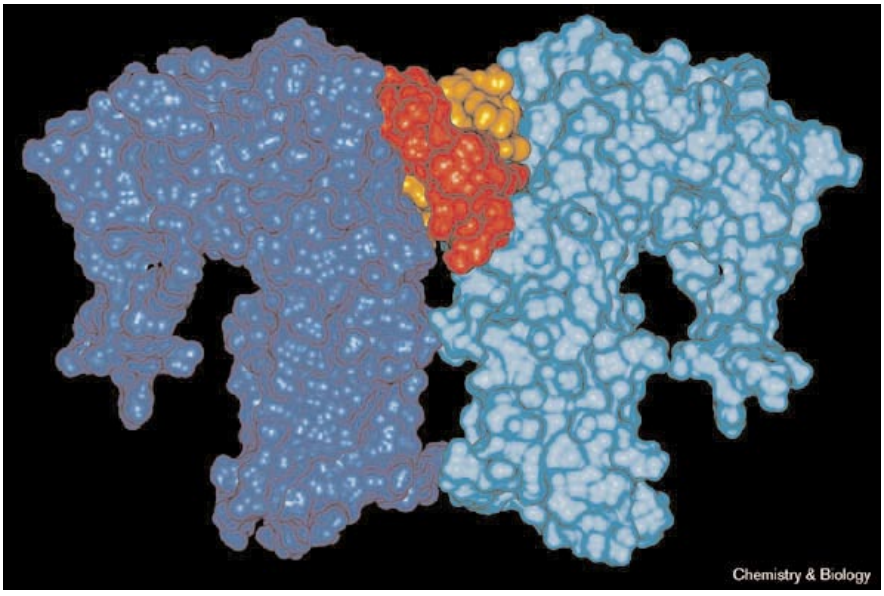
Peptides from phage display

About ten years ago, it was demonstrated that high-affinity ligands could be selected from peptide libraries displayed on bacteriophage [45–47]. Since then, quite a few successful applications have been described, many directed toward mimicking or antagonizing the function of a protein ligand [48–50]. The real power of the technique is that because the libraries can be very large (10^8 or more individual members), no assumptions need be made initially about how to bias the library. Those sequences that bind are amplified and can be identified easily by sequencing their encoding DNA. Further optimization often yields peptides of nanomolar affinity. Some targets (e.g. antibodies) yield peptides homologous to known ligands, whereas others yield entirely novel peptides whose affinity might never have been predicted.

A remarkable example of this latter class is an erythropoietin (EPO) agonist peptide that bears no resemblance to the natural hormone yet binds to the hormone site as a dimer, thus dimerizing and activating the receptor (Figure 5) [51,52]. A subtle change in the 20-residue peptide — substitution of 3,5-dibromotyrosine for a single tyrosine residue — converts the agonist into an antagonist [53]. Surprisingly, the crystal structure of the antagonist–receptor complex revealed not only the same 2:2 stoichiometry as the agonist–receptor complex, but also a shift in orientation between receptor monomers that may explain the shift to an antagonist [53].

Other antagonists of protein–protein interactions have been discovered using phage libraries. These include peptides blocking IL-1α binding to type I IL-1 receptor [54], insulin-like growth factor 1 binding to its regulatory binding protein IGFBP-1 [55], the angiogenesis factor VEGF binding to its cell-surface receptor KDR [56,57], IgG Fc binding to streptococcal Protein A [58], and HIV-1 gp120 binding to CD4 [59]. All of these peptides are between 10 and 20 residues in length, still a bit large to transform easily into small-molecule drug candidates. They are also quite hydrophobic and tend to cover a large surface on the target protein [52,57,58]. It will be a challenge to minimize these peptides without sacrificing their potency. A combination of peptidomimetic design with

Figure 5



Complex of the EPO-binding protein and the agonist peptide EMP-1. The complex contains two peptide molecules (orange and red) and two EPO-binding proteins (blue and light green; figure reproduced from [48]).

diversity methods seems most likely to balance these opposing forces.

Small molecules from screening

An exciting recent development is the identification of small molecules capable of blocking (or in some cases, promoting) cytokine receptor signaling. The first such molecule was designed to mimic arginine and phenylalanine sidechains of interleukin-2 (IL-2) important for binding to the α subunit of IL-2 receptor (Figure 6a) [60]. The design was not successful, because the mimic bound IL-2 itself; nevertheless, the molecule was a competitive inhibitor of IL-2 binding to its receptor (IC_{50} = 3 μM) and an important demonstration that such inhibitors could be obtained.

The next reported discovery was SB 247464 (Figure 6b), an activator of the receptor for granulocyte-colony-stimulating factor (G-CSF) [61]. This molecule was identified in a high-throughput screen, and chimeric receptor experiments indicate that it binds to the extracellular portion of the receptor. Maximal activity is seen at ~1 μM. Interestingly, SB 247464 self-antagonizes at about tenfold higher concentrations (see [15] for an extensive discussion of this phenomenon). This observation and the twofold symmetry of the molecule suggest that SB 247464 may bridge two receptor monomers; it would be interesting to know how much of the structure is required for the observed antagonism.

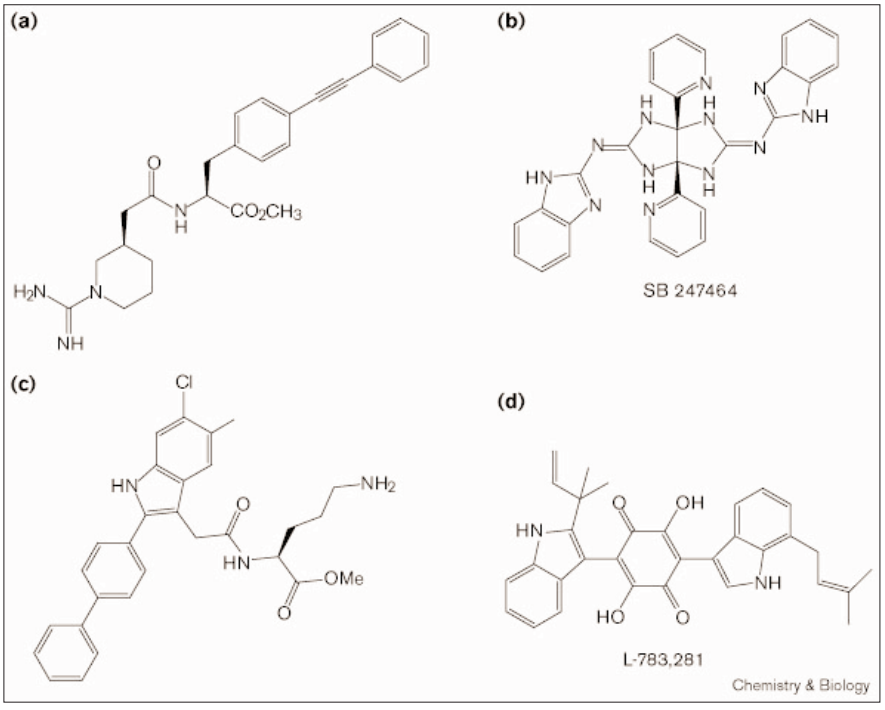
A third nonpeptide antagonist was discovered by screening a chemical library for inhibitors of human EPO binding to the extracellular domain of the EPO receptor [62]. A biphenyl indole derivative (Figure 6c) was found to inhibit the interaction with an IC_{50} of 60 μM; a multivalent synthetic intermediate acted as an EPO mimic.

The discovery of these antagonists is a real breakthrough, and additional examples are likely to follow. For those molecules identified through screening, it would be interesting to know more about the numbers and types of compounds screened and how many active molecules were found. Nevertheless, considering the molecules together (Figure 6), some similarities can be seen. All contain multiple aromatic rings, connected by rigid linkers that might be expected to prevent intramolecular hydrophobic collapse. This is also the case for the insulin receptor activator L-783,281 (Figure 6d). (This molecule binds to and activates the intracellular tyrosine kinase domain of the insulin receptor (EC_{50} = 3–6 μM) rather than competing for the insulin site [63]). The frequency of indoles is especially intriguing. It may be that such compounds are particularly well suited to binding protein surfaces.

Comparing strategies: HIV fusion inhibitors

Viral entry mediated by HIV gp41 involves a protein–protein interaction that has been the subject of much recent interest [11,64]. Crystal structures of a stable trimeric gp41 core domain (Figure 7a) [65–67] reveal a central coiled coil (N36) buttressed by helical segments from a more carboxy-terminal region of gp41 (C34). The six-helix trimer is believed to correspond to one of the species present during membrane fusion, probably during the later stages of the process, and it is the interaction of the carboxy-terminal helix with the surface of the coiled coil that has become the target of numerous studies.

Figure 6



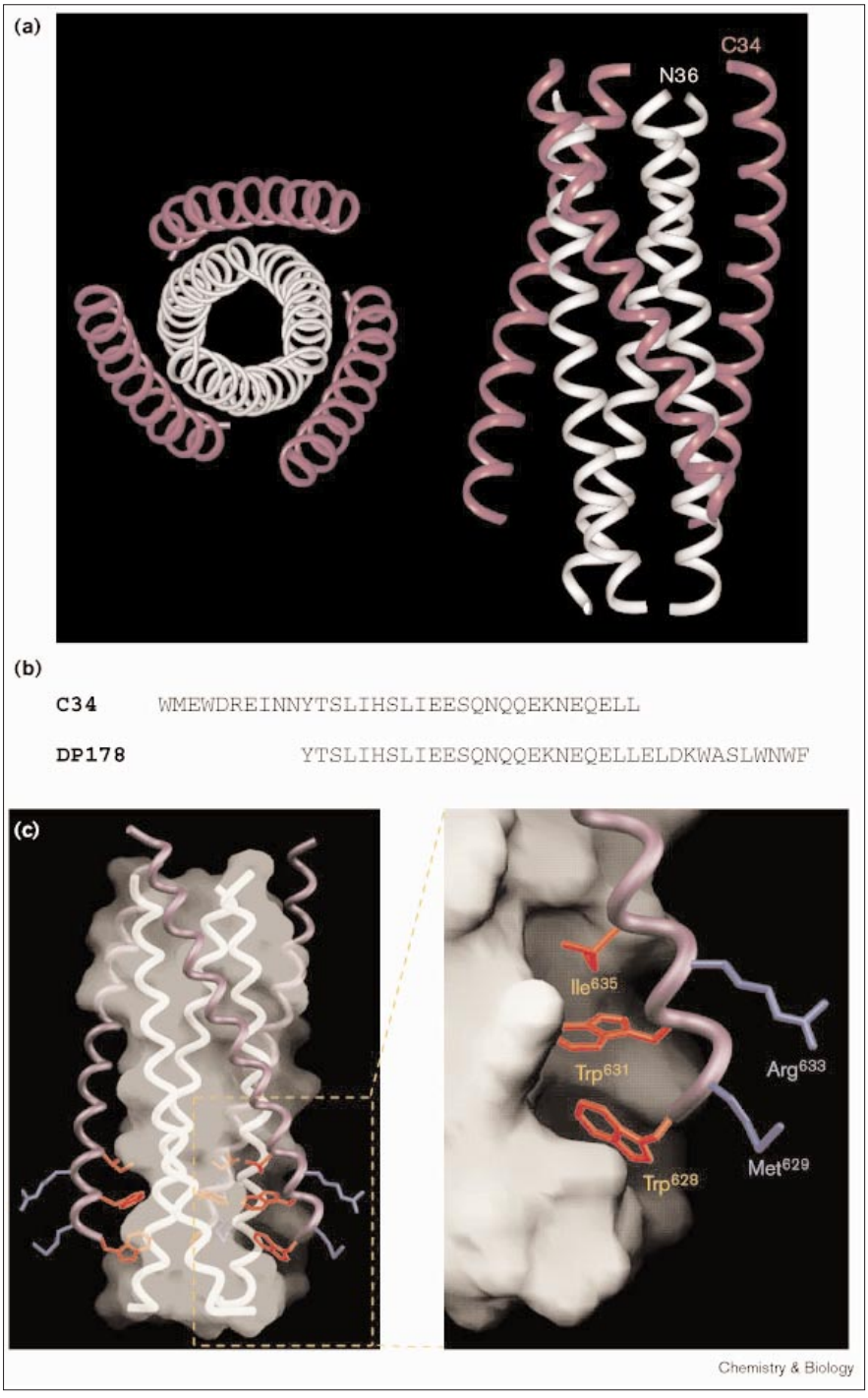
Small molecules that agonize or antagonize cytokine signaling. **(a)** IL-2 antagonist. **(b)** G-CSF receptor agonist (and antagonist, see text for more details). **(c)** EPO antagonist. **(d)** Insulin receptor agonist.

It was discovered several years ago that the linear 36-mer DP178 (derived from the gp41 sequence) could block viral fusion at nanomolar concentrations [68]. DP178 partially overlaps the carboxy-terminal helical segment seen in the crystal structures (Figure 7b), and it seemed likely that DP178 might bind in a helical conformation to an exposed coiled coil in gp41. Evidence supporting this model came from studies with truncated forms of DP178 that were cross-linked to enforce an α-helical structure [69]. Shortening DP178 to 27 residues abolished much of its potency, whereas introducing one or two helical locks progressively restored inhibitory potency of the 27-mer and increased peptide helicity, suggesting a relationship between helical structure and activity. Although the peptide with two locks was nearly as potent as DP178, the peptide could not be reduced much further in size [69]. A series of constrained 14-mers covering the entire helical region seen in the structures through the end of DP178 (48 residues total) failed to significantly inhibit viral infectivity, suggesting that an extended surface interaction is required for effective inhibition.

A further advance was made by Chan *et al.* [65,70] based on analysis of the gp41 crystal structure. Near the carboxy-terminal end of the central coiled-coil trimer, unusual interhelical pockets are present (Figure 7c); sidechains from one of the three exterior helices (Trp628, Trp631 and Ile635) are accommodated by each of these pockets. Substitution of any of these residues by alanine destabilized the structure of the soluble core domain (ΔT_m = 11–29°C) and reduced the inhibitory potency of the 34-residue peptide [70]. Introducing a series of hydrophobic residues at the position corresponding to Trp631 allowed the authors to correlate log IC_{50} values (from two different assays) with the melting temperature of the peptide complex. It appears, therefore, that inhibition by the 34-residue peptide requires not only that it adopt a helical conformation, but also that it form the complex with the central coiled coil of gp41. These authors further propose that the surface pocket of the central trimer may be a good target for a therapeutic small molecule; however, neither short linear peptides [70] nor helical constrained peptides [69] corresponding to the pocket-binding part of the exterior helix measurably inhibit viral infection. It seems, therefore, that the contacts between the pocket and its native binding partner do not provide sufficient energy upon which to build a small-molecule mimic.

However, two new efforts attempt to improve upon nature. In the first of these, an 18-residue segment from the gp41 outer helix (missing the residues that contact the surface pocket of the central trimer discussed above) was derivatized with non-natural building blocks added in three steps of split-pool synthesis, creating a library of 61,275 potential inhibitors [71]. The three non-natural elements were intended to fill the gp41 surface pocket in the place of the isoleucine and the two tryptophan sidechains. Positional deconvolution of the library yielded 3-cyclopentylpropionyl as the favored terminal

Figure 7



The gp41 core domain. **(a)** The amino-terminal peptide from gp41 (N36) forms a trimeric coiled coil, and the carboxy-terminal peptide C34 binds to the outer surface of the coiled coil [65]. **(b)** Sequences of C34 and the fusion inhibitor DP178 [68]. **(c)** Surface pocket in the N36 coiled-coil trimer (reproduced with permission from [70]).

group, followed by glutamic acid (coupled through the γ-carboxylate), *N*-(2-carboxyethyl)-4-aminomethylbenzoic acid, then the peptide. The hybrid peptide was unable to bind to the central trimer when cleaved from resin. However, the contribution of the non-natural elements to inhibitory potency could be determined by extending the peptide portion from 18 to 30 residues. The longer hybrid peptide inhibited cell fusion with an IC_{50} of 0.3 μM, compared with 6.6 μM for the unmodified 30-mer, and 2.7 nM for a 38-mer peptide that included the native isoleucine and tryptophan residues [71]. Therefore, although the non-natural elements do contribute some binding energy, the effect is modest, equal to ~40% of the contribution of the native sequence.

A quite different approach was reported by Eckert *et al.* [72] who developed a family of D-peptide inhibitors using phage-display methods. In order to target the surface pocket of the central gp41 coiled coil directly, they designed a chimeric trimer in which a very soluble and stable coiled coil (based on GCN4) was fused, in heptad register, to 17 gp41 residues; these gp41 residues include all those needed to define the pocket. Using this engineered target (synthesized in D-form), 12 phage clones were ultimately selected. Nine of these appear to bind specifically to the pocket region of the target and define a family of 16-mer peptides with a consensus sequence of $GACX_5EWXWLCAA$. IC_{50} values for the nine peptides (now synthesized in D-form) range from 3.6–130 μM. A crystal structure was determined for one of the bound peptides [72]. The peptide does bind to the pocket region of the target: the provocative observation is that the consensus tryptophan residues and leucine of the peptide are presented on the surface of a helix, occupying roughly the same positions as the tryptophan residues and isoleucine of the native gp41 helix (although the sidechain orientations do differ).

Considering all these studies together, one may conclude that despite the observed surface pocket in the gp41 trimer the binding energy in the native complex is distributed rather evenly along the interhelical surface. Nevertheless, a binding selection can yield peptides that resemble the native sequence in some key respects, but have a larger interaction energy with the surface pocket. Again, this highlights the power of phage display to suggest solutions that would have been difficult to access through structure-aided design.

Future prospects

There have been many exciting developments that bring small-molecule inhibitors of protein–protein interactions closer to practical use. Much work remains, however, to define which strategies will be most practical for discovering such molecules, and which proteins will be the best small-molecule targets. The very recent reports of the first genome-scale protein–protein interaction maps (for yeast) [73,74] suggest that biological chemists and structural biologists will have an abundance of interesting possibilities to explore.

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